APPLICATION FOR LETTERS PATENT

Title: MIMICKING THE METABOLIC EFFECTS OF CALORIC RESTRICTION BY ADMINISTRATION OF GLUCOSE ANTIMETABOLITES

Inventors: Josef Pitha and George Roth

This is a continuation-in-part of the application Ser. No. 08/889,877 filed July 8, 1997, now pending.

Field of the Invention:

This invention relates to the use of glucose anti-metabolites to alter utilization of glucose or other energy sources and to mimic metabolic effects of caloric restriction.

Background of the Invention:

Biological theories correctly predict the finding that a restriction of caloric intake by food deprivation slows down certain undesirable cellular processes in laboratory animals, many associated with aging and age-related diseases.

It is also known that hyperinsulinemia is a risk factor associated with several such disease processes, including heart disease and diabetes (Balkau and Eschwege. Diabetes Obes. Metab. 1 (Suppl 1): S23-31, 1999). The avoidance of hyperinsulinemia should be a goal for treatment of many individuals.

Glucose anti-metabolites such as 2 deoxy-D-glucose are compounds related to glucose. However, due to structural differences from glucose such compounds block or inhibit certain aspects of carbohydrate metabolism (Rezek, et al., J. Nutr. 106:143-157, 1972). These anti-metabolites exert a number of physiological effects, including reduction of body weight,

decrease in plasma insulin levels, reduction of body temperature, retardation of tumor formation and growth, and elevation of circulating glucocorticoid hormone concentrations. (For a review see Roth et al., Ann. NY Acad. Sci. 928: 305-315, 2001.) These effects result from inhibition of carbohydrate metabolism. Reduced insulin levels and body temperature are two of the most reliable indicators of this altered metabolic profile (Masoro et al., J. Gerontol. Biol. Sci. 47:B202-B208, 1992; Koizumi et al., J. Nutr. 117: 361-367, 1987; Lane et al., Proc. Nat. Acad. Sci. 93:4154-4164,1996). Intervention designed to provide beneficial physiological regulation of biological processes while allowing animals to avoid undesirable effects of caloric restriction would provide improved health benefits.

Summary of the Invention:

It is the purpose of this invention, to provide a means of mimicking the beneficial metabolic effects of caloric restriction by carefully controlled administration of anti-metabolites of glucose. Some preferred antimetabolites for use according to the teachings herein include ketoses (mannoheptulose) and anhydro-sugars (anhydroglucitols and anhydromannitols) that are structurally similar to glucose. Using methods of the invention, it is possible to obtain beneficial biological results associated with caloric restriction comprising administration of a composition containing at least one active agent which blocks use of glucose as a source of energy in cells in amounts sufficient to lower tissue glucose level and decrease in plasma insulin levels in the

non-diabetic animal.

Description of the Invention:

It is the purpose of this invention to provide benefits associated with caloric restriction by controlled administration of antimetabolites of glucose. Judicious use of compounds that block the normal metabolism of cellular glucose can result in changes in physiological function that are similar to those arising from caloric restriction. The compounds and compositions used in accord with the teachings herein often lower body temperature. Such lowering of body temperature and slowing of the rate of metabolism in the tissues often is beneficial in treatment of trauma and in other treatment modalities where decrease in metabolic rate is desirable.

Two related aspects must be addressed. Glucose is used by cells both as an energy source (catabolic mode) and for incorporation into other compounds (anabolic mode). Inhibition or interference with anabolic uses of glucose should be avoided, since this may lead to production of anomalous glycoproteins and glycolipids and eventually to undesired side effects. It should be noted that various non-nutritious sweet compounds (some of them carbohydrates) have been suggested as agents to reduce obesity based on the theory that, if these compounds can not be a source of energy, caloric intake may be reduced. The instant invention does not relate simply to agents that lack nutritional value. These prior art agents that have been used simply to avoid/treat obesity perform a different function and do not

provide the benefits sought in the practice of the instant invention.

Decreased utilization of glucose as energy source by 2-deoxy-D-glucose:

To fully mimic the beneficial effects of caloric restriction, it is necessary that glucose anti-metabolites be given over an extended time period. Previous studies clearly show that it is not possible to administer compounds such as 2- deoxy-D-glucose in high doses, since significant untoward side effects and toxicity have often been observed. However, studies in rodents (Lane et al., J. Anti-Aging Med. 1 (4):327-337, 1998) have shown that long-term disruption of glucose metabolism using a lower dose of 2 deoxy-D-glucose can mimic some of the major metabolic hallmarks of caloric restriction, including reduced body temperature, weight loss, and lower fasting insulin levels.

In light of the above potential physiologic benefits of caloric restriction weighed against the negative aspects of metabolic inhibition by 2-deoxy-D-glucose, alternatives which act as antimetabolites of glucose without the potentially harmful side effects are preferred for purposes of practicing the invention.

Decrease of availability of glucose to cells by 5-thio-D-glucose.

5-Thioglucose, an analog of glucose, has (in vivo) more pronounced effects than 2-deoxy-D-glucose. The compound is believed to act mainly by inhibiting glucose uptake by cells.

The majority of 5-thioglucose (97%) injected into a rat has been found excreted unchanged in urine (Hoffman et al., Biochemistry 7, pp 4479-4483 (1968)). 5-Thioglucose is remarkably non-toxic; LD_{50} was measured to be 14 g/kg, by injection, in rats (Chen et al., Arch. Biochem. Biophys., 169, pp 392-396 (1975)).

Since 5-thioglucose seems to be excreted unchanged in urine, this compound presents certain advantages for chronic administration over 2-deoxy-D-glucose. Nevertheless, since 5-thioglucose inhibits glucose uptake, appropriate dosing can result in benefits associated with caloric restriction.

Effects of 3-O-methylglucose

This analog of glucose, in contrast with 2-deoxy-D-glucose, is not metabolized (Jay et al., J. Neurochem. 55, pp. 989-1000 (1990)) and, thus, may provide certain advantages for use in chronic administration. In the context of this invention, 3-O-methylglucose can prevent utilization of glucose as an energy source as demonstrated by response to its administration in rats. The responses were about seven times weaker than those to 2-deoxyglucose.

Effects of anhydrosugars: 1,5-anhydro-D-glucitol (polygalitrol):

This compound is a non-reducing analog of glucose and is enzymatically converted to 1,5-anhydroglucitol-6-phosphate, albeit the conversion is less efficient than that of 2-deoxy-glucose (Sols et al., J. Biol. Chem., 210, pp 581-595 (1954)). 1,5-anhydroglucitol-6-phosphate is an allosteric (non-competitive) inhibitor of hexokinase, which catalyzes the first and the

regulatory step of the entire glycolysis (Crane et al., J. Biol. Chem., 210, pp. 597-696 (1954)). Furthermore 1,5 anhydro-glucitol-6-phosphate is a non-reducing analog and cannot be a substrate for the next step of glycolysis catalyzed by glucose 6-phosphate isomerase. Consequently, this analog could accumulate in cells and act as a very effective metabolic block to glucose utilization. Another advantage relating to its non-reducing character is that this compound cannot be incorporated into glycolipids, glycoproteins and glycogen. Thus, its effects are specific to glycolysis and would not be expected to affect other metabolic processes or exert toxicity of some glucose anti-metabolites previously discussed.

Interestingly, this compound (or its phosphate) has been found in the human body. It was found to be present in cerebrospinal fluid of patients who had occasional high blood glucose (from diabetes and diseases of kidney) in large enough concentrations to be detected in tests performed in normal clinical settings.

Use of 2,5-anhydro-D-mannitol and 2,5-anhydroglucitol:

These compounds are non-reducing analogs of fructose. Fructose is an important component of food and fructose phosphates and diphosphate are intermediate products of glycolysis. Nevertheless, inhibition of metabolic events involving fructose and its phosphates by anhydrosugar analogs is difficult. Alpha and beta anomers of fructose, which spontaneously inter-convert, correspond to different anhydrosugars, to 2,5-anhydroglucitol and

2,5-anhydromannitol, respectively. Thus, only a few of the enzymatic conversions can be inhibited by a single compound. The 2,5-anhydromannitol has been investigated in some detail. That compound is taken up by cells and converted into 2,5-anhydromannitol-1-phosphate. That phosphate is an analog of fructose-1-phosphate, but can not be cleaved by the aldolase and, therefore, the utilization of both glucose and fructose by cells is blocked. The 2,5-anhydromannitol had been found to interfere in glucose formation and utilization in isolated rat hepatocytes (Riquelme et al., Proc. Natl. Acad. Sci. USA, 80, pp 431-435 (1983)).

Decrease of glucose utilization as energy source by ketoses.

Mannoheptulose is present in reasonable amounts in some foods (e.g. some avocados contain up to 5% of the wet weight) and can be classified as a "generally recognized as safe" substance for the human consumption. In studies of metabolism, 10 grams of mannoheptulose have been safely administered to humans orally. About 5% of the mannoheptulose ingested was reported to appear in urine after oral dosing. The fate of injected mannoheptulose has previously been investigated in rats: 66% was excreted unchanged, 29% was metabolized, and, a day after the injection, 5% remained in the body (Simon et al., Arch. Biochem. Biophys., 69, pp. 592-601 (1957)).

Example I

Preparation of mannoheptulose-containing supplement:

Fresh avocados (Lula variety) were obtained from Fresh King

Incorporated (Homestead, Florida). The avocados were manually split open and the pits were removed and discarded. The remaining skin and pulp were ground through a Hobart Commercial Food Preparation machine (serial # 11-10410235) using a 12¼ sieve. The ground avocado was then transferred to an Edwards Freeze Drier (Super Modulyo Model, Crawely, Sussex, England). The freeze drier was set at -20°C for the first 24 hours, -5°C for the following 24 hours and 5°C for the final 72 hours. Upon removal from the freeze drier, the meal was ground to a powder using a Straub Grinding Mill (model 4E, Philadelphia, Pennsylvania). The avocado meal was analyzed and found to contain 10.35% mannoheptulose. (It should be noted that the amount of mannoheptulose found in avocados varies with the particular strain, some avocados having little or no mannoheptulose.)

Example II

Administration of mannoheptulose to beagle dogs:

The use of mannoheptulose for purposes of obtaining benefits associated with inhibiting metabolism of glucose was tested in beagle dogs. A total of 12 beagles were utilized for the study and were fed a standard commercial diet throughout the study period. Fasting blood samples were drawn 7, 6, 4, and 2 days prior to administration of mannoheptulose. The mannoheptulose was delivered to the dogs in the form of a freeze-dried avocado meal containing 10% to 12% mannoheptulose. This preparation was adjusted to provide mannoheptulose doses of 2, 20, and 200 mg/kg body weight (MH-2, MH-20, MH-200, respectively). Fasting blood

samples were collected 1, 3, 5, and 7 days after initiation of the administration of mannoheptulose.

Results

Insulin levels were lowered by up to 35% in dogs who had received the avocado meal when compared to those dogs on similar diets who had not received meal with their diets. Those changes were similar to the decreases found in mammals on caloric restricted diets. In contrast, plasma glucose concentrations of dogs fed the same standard diet which did not contain the avocado meal did not show such effects.

The mechanism by which insulin is reduced relates to the fact that glucose must be metabolized by the pancreas to stimulate insulin secretion (German et al., Proc. Nat. Acad. Sci. 90:1781-1785. 1993). Mannoheptulose is thought to inhibit glucokinase, the initial enzyme involved in glucose metabolism in pancreas and liver. Therefore, reduced insulin levels indicate that mannoheptulose has indeed inhibited glucose metabolism. This effect on glucokinase by mannoheptulose would indicate use of mannoheptulose directed at inhibition of tumor growth as an alternative to administration of 2- deoxy-D-glucose. (See Board, M., et al., Cancer Res. 55(15): 3278-3285. 1995.) Mannoheptulose would present a safe alternative to 2-deoxy-D-glucose, since it would avoid some untoward effects seen when 2-deoxy-D-glucose is administered on a long-term basis.

The availability of glucose to cells can also be decreased using other dietary supplements than those specifically identi-

fied herein which have similar effect on metabolism of glucose that can result in an inhibition of glucose processing.

The methods of the invention may be practiced by administering the active agents orally or parenterally, though oral administration would be the norm. When lowering of tissue metabolism is desired, as an adjunct to treatment of trauma, the active agents may be administered intravenously.

Dosage will depend on the agent used and will vary depending on the extent of lowering of tissue metabolism that is desired and the size and condition of the animal to which the agent is to be administered. Dosage in the range of .001 g/kg to about 1 g/kg would be suggested. Dosage at the lower range would be appropriate when using 2-deoxy-D-glucose in large mammals. Higher dosage, particularly of compounds such as 5-thio-D-glucose or mannitol should be readily tolerated.